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ENZYMIC METHOD FOR OXIDATIVE DEGRADATION OF FLUORESCENT COMPOUNDS

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[Abstract]

Fluorescent compounds in aqueous systems can be degraded by treating such aqueous systems with an enzyme from the group of the oxidases and peroxidases and an oxidation agent from the group of O_2, H_2O_2 and O_2 or H_2O_2-containing or forming compounds at 5-90°C, and a pH value from 5-10. The treatment can be carried out in the presence of surface-active auxiliary substances.

Description

The invention concerns a method for enzymic degradation of fluorescent compounds in aqueous systems, in which such aqueous systems are treated with one or more oxidases or peroxidases and O_2, H_2O_2 or compounds that form O_2 or H_2O_2.

Fluorescent compounds are mainly used as optical brighteners. These are substances that absorb UV light, for example the portion of daylight at about 280-430 nm, in solution or on a substrate and emit the absorbed energy as blue fluorescence light of wavelength of about 400-550 nm. Textiles, paper, cardboard or pasteboard appear whiter and brighter after treatment with optical brighteners, because a part of the region of the daylight spectrum that is not perceptible to the eye is converted to visible light. Brighteners absorb in the UV range or reduce the reflection in this range. At the visible wavelengths, mostly at a peak of 430-450 nm, they increase the reflection through the emitted fluorescence. The whiter the substrate is, the more effective the brighteners are. However, they are not a substitute for bleach. The industrially important brighteners are compounds whose molecular structure contains 4,4'-diaminostilbenedisulfonic acid, distyrylbenzene, distyrylbiphenyl, stilbenyl-2H-triazole, certain benzoxazoles, benzofurans and coumarins as backbone.

Optical brighteners are used in many ways. For instance, brighteners are added to detergents, in order to replace brightener components that are present in textiles that can be eluted out in the washing process because of poor fixing to fibers. Batchwise or continuous processes analogous to the known dying processes are used in the industrial conduct of such washing operations.

In addition, optical brighteners are used in the production of coated and uncoated papers, especially printing papers. The degree of whiteness here can be considerably increased through the use of very small amounts; brighteners act with the greatest efficiency in the concentration range of 0.002-2.0 wt% with respect to the substrate, and are supposed to be distributed on the substrate as far as possible in a monomolecular layer. In papermaking brighteners can be added directly to the pulp suspension from which the paper is formed by dewatering. However, the most common use in practice is in the paper surface, where the brightener is applied, for example, via a size press, together with other auxiliary agents like surface sizing agents or starch,

to the paper surface. Another important area of use is the addition of brighteners to paints. In the paper industry, cheap triazinylaminostilbenes or distyrylbiphenyls are preferably used.

Environmental and safety aspects have long been given considerable consideration in the development, production and use of optical brighteners, for example in liquid or powder formulations. For instance, anionic brighteners, especially ones with sulfonic acid groups, were developed so that they behave substantively toward cellulose and become fixed so that they are bleedfast.

For use in food packaging papers it is required that the brightener not migrate to the foodstuff, i.e., that a score of 5 is achieved in the bleed test in accordance with DIN 53.991, sheet 2. This is similarly true for hygienic paper (DIN 53.991, sheet 4).

For some time increased bleeding of optical brighteners or other fluorescent compounds has been increasingly seen with paper and cardboard to which brighteners have not been added have been added only in very low amounts; this is clearly connected with the increased use of secondary fibers, whose cellulosic structure has been damaged and for this reason no longer contains the originally present optical brighteners in a firmly bound form. This is a problem in particular for manufacturers of foodstuff packaging papers, since in this field it is necessary to pass the bleed test with a score of 5 for permission. (Scale from 1-5; higher values show stronger resistance to bleeding out; method DIN 53.991, sheet 2 and sheet 4.). Besides the recycling of brightened papers, a possible source is presumed to be the replacement of chlorine bleaches in pulp manufacture by TCF bleaches (TCF = totally chlorine free). In addition, the effect of deinking chemicals on the degree to which brighteners continue to be fixed is largely unknown. Also, the behavior of optical brighteners in recycling has still not been thoroughly investigated. In the field of waste paper recycling, for example, one would need to achieve a situation where the brightener remains firmly enough fixed to the fiber or becomes fixed right from the start in a suitable way in order to prevent bleeding out. Through this one could ensure that even originally brightened secondary fibers could be used as raw materials for papers that were not then to be brightened, without producing a disadvantageous effect. If fluorescent compounds that derive from pulp production or brighteners from secondary fiber materials are not sufficiently fixed in paper by mechanical or chemical modification, the use of certain paper types for packaging of foodstuffs will be precluded, since in this case they will no longer pass the bleed test.

Besides the presence of brighteners in paper and the related discharge of these brighteners from the production cycle of a paper plant there is also the possibility of removing the brightener after it has been desorbed from the fibers by treating the wash water or flotate from the paper pulp cycle. However, it is hardly possible to control the subsequent presence and possible degradation of brighteners, because of the conventional conditions currently in practice and the use of different chemicals in different concentrations. Optical brighteners were earlier

degraded in Mt paper* processing by hypochlorite. However, this produced organochlorine compounds that besides having an unfavorable effect on the toxicological rating increase the AOX content of the wastewater in every case. Another method of decomposing optical brighteners is oxidation by persulfate in a strongly alkaline medium. Disadvantages of this treatment method are the high chemical requirement and stress on the environment due to salts that remain.

Furthermore, it is possible to quench the fluorescence of the brighteners by cationic components such as polyamidamines, polyamidamine-epichlorohydrin resins, dicyanodiamide condensates or other fluorescence quenchers. In this case the fluorescent compound is not broken down, but rather made inactive through complexing.

Other problematic wastewaters are formed in the home or commercial washing of textiles, since the washing agents that are used, as noted above, contain various optical brighteners, which are intended to replace any brighteners washed out of the textiles, but which do not entirely remain in the textile as intended, but rather appear in the wastewaters from the washing machines.

Brighteners that are contained in the wastewaters from private households or commercial plants, especially from the paper, textile and detergent industry, are very difficult to break down in biological wastewater treatment processes. They are largely adsorbed in the sewage sludge, which in turn must be disposed of at high cost.

Besides the problem that was mentioned in the recycling of waste paper and with various other wastewaters, a problem can also arise in the production of brightened paper types and cardboard in that because of the pressure to avoid waste and to deal with resources like fibers, auxiliary substances and water gently, one would wish to keep the required water cycles closed, but one must expect the accumulation of brighteners, since it is not possible to guarantee complete absorption of the brighteners on the cellulose fibers. A further increase of the amount of brightener in the water cycle can result from the use of brightened waste material.

An extraction of brightener residues from the sieve water of paper machines is not possible with the currently available methods. The problem of the accumulation of optical brighteners in the water cycles of the paper industry is magnified further through the greatly increasing demand for brightened papers, with additionally elevated demands on the degree of whiteness.

For this reason there is a great need for an environmentally friendly method for removing optical brighteners, as the most important representatives of fluorescent compounds in wastewater treatment (wastewaters from various sources, as presented above), in the control and

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* [Translator's note: Term ("Mtpapier") not found in my sources.]

supervision of closed cycles, and in various uses of optical brighteners in the paper, textile and detergent industry. The problem is particularly important for the paper industry because of the significantly rising quotas for waste paper recycling, which at the present time are about 30-50% in Europe.

Therefore, the task of the invention is to make available a method that degrades fluorescent compounds so that subsequent biological wastewater treatment is possible. The method in accordance with the invention is to be applicable also to TCF pulp, in which up to now only oxidative removal of fluorescent compounds, for example with chlorine or hypochlorite, has been possible. In addition, the disadvantages of the known methods with their high chemical use and the formation of chlorine-containing compounds are to be overcome; for example, in the case of persulfate decomposition high pH values must be established, which again leads to a high salt load after the required neutralization. Besides the said chemical and toxicological disadvantages, the use of these chemicals is expensive. Such methods, therefore, need improvement from the standpoint of economy.

It was found that oxidases and peroxidases are suitable for decomposing fluorescent compounds, for example optical brighteners in wastewaters and water cycles that contain such fluorescent compounds, using O_2 or H_2O_2.

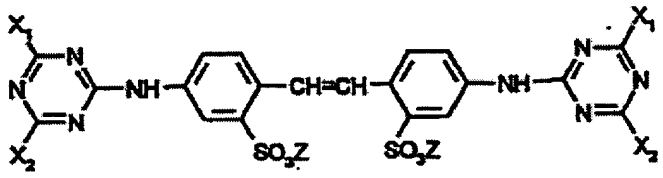
Some applications of peroxidases have already been proposed: For instance, it is known from WO 89/09813 that peroxidases can be used for oxidation of phenol bodies. In addition, peroxidases are proposed for bleaching wood pulp, for treatment of wastewaters of the pulp industry or for use in textile laundry, in the latter case in particular to prevent backstaining. EP 406 617 proposes ligninases in combination with xylanases for bleaching of paper pulp. Enzymes for degradation of lignin are claimed in EP 429 422. In accordance with US 5,370,770 soybean peroxidase is used in the deinking of used paper. The decomposition of phenolic contaminants by means of peroxidases is also proposed in US 5,178,762.

The use of oxidases and peroxidases for enzyme-catalyzed oxidative decomposition of fluorescent compounds, for example optical brighteners, has clearly not been accomplished up to now, in any case not described, although the problems mentioned above are pressing and known.

The invention concerns a method for oxidative decomposition of fluorescent compounds present in aqueous systems, which is characterized by the fact that such aqueous systems are treated with at least one enzyme from the group of the oxidases and peroxidases, which can be present by themselves as such or combined with at least one other enzyme with hydrolytic activity, and with an oxidation agent from the group consisting of O_2, H_2O_2, and mixtures that contain or form O_2 or H_2O_2 at a temperature from 5-90°C, and a pH from 5-10, where the treatment is carried out in the absence or in the presence of surface-active auxiliary substances.

Aqueous systems in the sense of the invention are wastewaters from the production or the treatment of textiles, fiber articles like paper, cardboard or pasteboard, the underlying fibers and polymers, especially ones based on cellulose, also aqueous dispersions or suspensions of such textile fiber structures, fibers, and polymers. The aqueous system in accordance with the invention can, furthermore, be a treatment bath, for example a textile treatment bath, preferably one or clothing textiles. Fiber structures like paper, cardboard or pasteboard, in particular brightened, coated or uncoated printing and writing papers, are usually refined in a pulping machine when they are recycled, freed of coarse contaminants, and then subjected to a washing or flotation deinking. The enzymic treatment to decompose optical brighteners in accordance with the invention can take place before, during or after the dissolving of the printing ink components. The thus purified pulp is then frequently subjected to an additional bleaching step, where enzymes can also be used. For the case where the optical brightener is absorbed on the fiber, but [the fiber] is to be used for production of unbrightened paper, cardboard or cartonage in a recycling process, it can be advantageous to use other enzymes that cause, for example, the desorption of the optical brightener from the fiber, in addition to the oxidases or peroxidases.

The method in accordance with the invention for oxidative decomposition with the aid of enzymes from the group of the oxidases and peroxidases can be applied to basically all fluorescent compounds, especially ones that are used as optical brighteners. A large portion of such fluorescent compounds contain 4,4'-diaminostilbenedisulfonic acid, distyrylbenzene, distyrylbiphenyl, stilbenyl-2H-triazole, benzoxazole, benzofuran or coumarin as molecular backbone, as noted above. Of these fluorescent compounds, ones that contain 4,4'-diaminostilbenedisulfonic acid or distyrylbiphenyl as molecular backbone are particularly important. Fluorescent compounds that contain the 4,4-diaminostilbenedisulfonic acid backbone can be subjected to the method in accordance with the invention with special preference. Important examples of fluorescent compounds are, for example, ones of the formula



(I),

in which

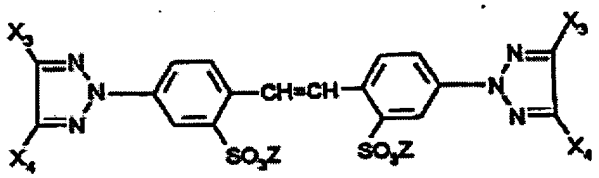
X_1 means amino, methylamino, ethylamino, dimethylamino, diethylamino, 2-hydroxyethylamino, 3-hydroxypropylamino, di-(2-hydroxyethyl)amino, di-(2-

hydroxypropyl)amino, 2-sulfoethylamino, morpholino, anilino, chloranilino, sulfanilino, methylanilino, or disulfoanilino, and

X_2 means hydroxy, methoxy, ethoxy, methoxyethoxy, chlorine, or X_1,

Z is an alkali metal, amine, or ammonium ion,

and those of the formula



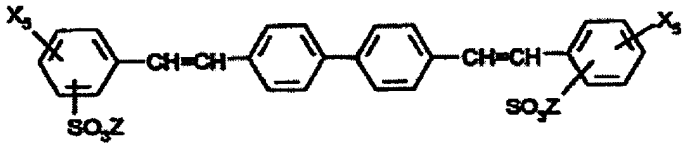
(II),

in which

X_3 and X_4 mean hydrogen, methyl, ethyl, phenyl, or sulfophenyl,

Z has the meaning given above,

and of the formula



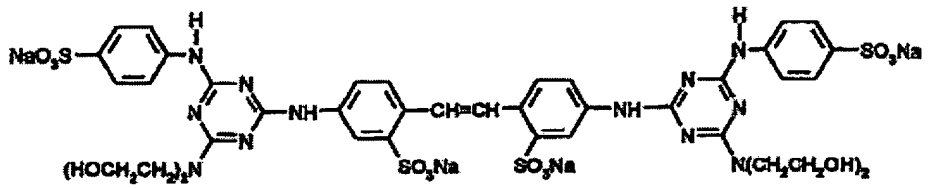
(III),

in which

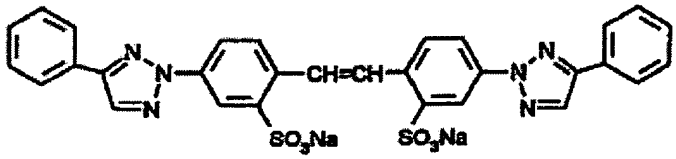
X_5 means hydrogen, methyl, ethyl, methoxy, ethoxy, chlorine, or sulfo, wherein all cases

Z is an alkali metal, amine, or ammonium ion.

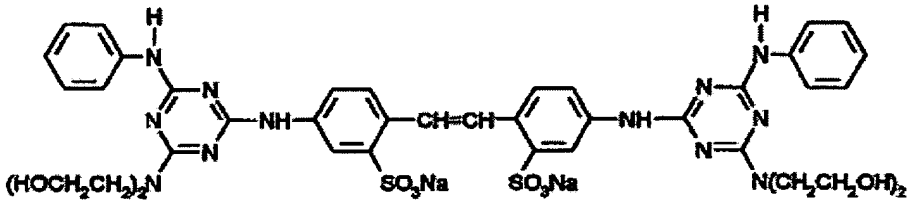
Such fluorescent compounds are known, for example, from EP 409 028. Important individual examples of these are those of the following formulas (A) through (G), which in part are products of Bayer AG and sold under the trade name Blankophor®:



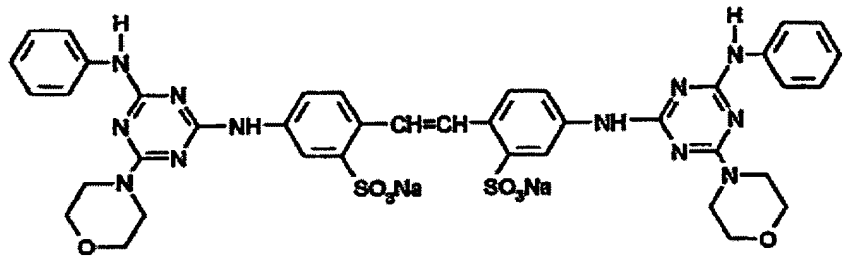
(A)



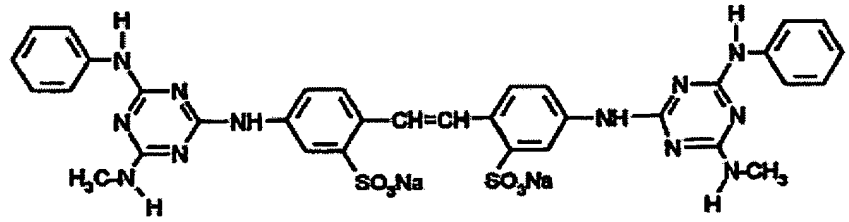
(B)



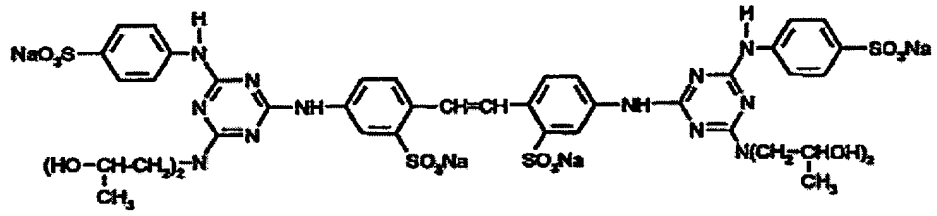
(C)



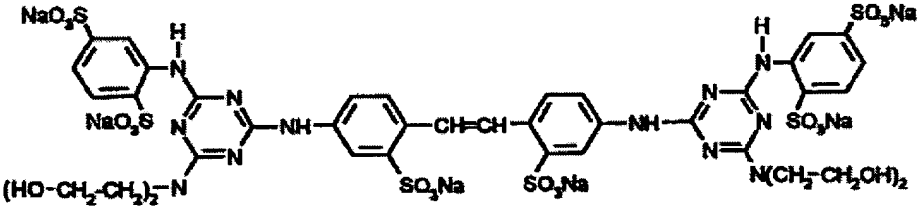
(D)



(E)



(F)



(G)

Suitable for use in the method in accordance with the invention are enzymes from the group of the oxidases and peroxidases, which according to "Enzyme Nomenclature 1984, Academic Press Inc., New York, London (E.C.)" can be classified under the larger group of the oxidoreductases, which catalyze electron transfer between various substances. Examples of such enzymes, which are characterized in E.C. by numerical combinations and in this way can be found there, are mentioned below:

Peroxidases (E.C. 1.11.1.7)

from plants, microorganisms, or fungi, like horseradish peroxidase, soybean peroxidase, or a peroxidase from Coprinus, for example Coprinus cinereus or Coprinus macrorhizus, or Bacillus, for example Bacillus pumilus or Bacillus myxococcus, Trametes, Rhizoctonia, Pseudomonas, Streptomyces, Candida, Curvularia, Cercospora, Polvporus pinsitus, Lignin peroxidases, Mn-dependent peroxidases, peroxidases from Phanerochaete, Basidiomycetes. In addition, the peroxidases with improved stability from WO 93/24618, chloroperoxidases, and lactoperoxidases should be mentioned.

Oxidases (E.C. 1.10.3.1)

Cellobiose oxidase from Phanerocaete chrysosporium or Humicola, glucose oxidase, catechol oxidase, polyphenol oxidase. The oxidases also include the laccases (E.C. 1.10.3.2), in particular laccases from Trametes versicolor, Phanerochaete chrysosporium; such enzymes are produced, for example, by Polyporus pinsitus (Trametes villosa).

The peroxidases are used together with H_2O_2 or a compound that contains or forms H_2O_2 as oxidation agent. Besides H_2O_2 itself, most often in the form of various highly concentrated and commercially available aqueous H_2O_2 solutions, one may mention in this regard: perborates, persulfates, percarbonates, peresters, peroxides, Fenton's reagent, peracids or enzymic systems that form H_2O_2 such as glucose/glucose oxidase/oxygen, anilinic acid-oxidase, ureate oxidase, cholesterol oxidase, amine oxidase or alcohol oxidase. Oxidases, including the laccases, are used with O_2 or compounds that contain or form O_2, or O_2 sources.

It can be advantageous to use, in addition to the said enzymes and oxidation agents, other auxiliary agents during the treatment such as metal salts (for example, alkali metal halides, sulfates, phosphates, acetates, etc.), saccharides (for example, cellobiose, glucose, fructose, ribose, mannose, galactose, arabinose, trehalose, xanthane), halide ions, buffer solutions (for example, phosphate, borate, acetate buffers), and reducing or oxidation agents (for example, $NaHSO_3$, $Na_2S_2O_4$, or formamidinosulfinic acid, or perborate, periodate or percarbonate). These auxiliary agents can be suitable for controlling the electrochemical potential of the mixture.

It can be additionally advantageous to use substances to accelerate the activity of the peroxidases or oxidases. The effect of such accelerators is due to the fact that short-lived radicals or other oxidized states are formed in the reaction system and can assist in the oxidative decomposition of the fluorescent compounds. A stable oxidized primary product acts as electron acceptor, with the electron acceptor having a half life that is greater than the turnover number of the oxidation of the accelerator, as presented, for example, in WO 94/12619. Such accelerators are, for example, azines of the formula A = N – N = B (WO 94/12620), also N-methylphenothiazine [sic], 3,3',5,5'-tetramethylbenzidine, 4-amino-4'-methoxystilbene, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate). Metal ions, halide ions, phenol, p-

hydroxybenzoic acid, p-hydroxybenzene sulfonate, p-hydroxycinnamic acid, 7-hydroxycoumarin, guajacol, or vanillin can also be used as accelerators.

The said enzymes from the group of the oxidases and peroxidases can be present both by themselves as such, as well as combined with at least one other enzyme that has hydrolytic activity. This latter case has the advantage that the enzyme preparations obtained from the sources mentioned above (plants, microorganisms, or fungi), which are frequently a mixture of several enzymes, do not have to be separated into the individual enzymes. Additional costs are avoided through this. Such other enzymes that can be combined with the oxidases and peroxidases are, for example, hydrolases like cellulases, for example from Phanerochaete, Humicola, Fusarium, Pseudomonas, Trichoderma reesei, or Aspergillus; hemicullulases, preferably xylanases, like Pulpzyme HC®; lipases, for example from Pseudomonas, Humicola, Candida, Chromobacter, Aspergillus; proteases, for example NUE 0.6 MPX, Aquaderm, Pyrase®, Alcalase®, Esperase®, Savinase® (products of Novo Nordisk); amylases like Denimax® and others; pectinases, dehalogenases, and pullulanases. Such other enzymes are mentioned, for example, in WO 91/17243, WO 91/17244, WO 91/10732, US 5,338,403, WO 94/23053 and WO 91/14019.

It can additionally be advantageous to mobilize fluorescent compounds, for example, brighteners, that are adsorbed on brighteners, through the co-use of surface-active auxiliary agents. Anionic, cationic, or nonionic, as well as amphoteric surfactants are possibilities as such surface-active compounds. Examples of these are salts of fatty acids, ammonium salts of fatty amines, polyethers with long-chain molecular residues, where the polyethers are formed through ethoxylation and/or propoxylation, partial fatty acid esters of glycerol, trimethylolpropane, pentaerythritol, oleic acid sarcosides, sulfosuccinates, polyacrylic acid derivatives with hydrophobic side chains or end groups, polyamidamine emulsifiers, etc. Such surface-active auxiliary agents have long been known to the specialist.

The method in accordance with the invention is carried out at a temperature of 5-90°C, preferably 10-60°C, especially preferably 20-25°C, and in the pH range from 4-10, preferably from 5-8. The residence time of the aqueous system to be treated within the scope of the method in accordance with the invention is dependent on many factors, for example the concentration of the fluorescent compound, the concentration of the enzyme, the concentration of the oxidation agent, the temperature, and the pH value. In many cases a maximum time of 200 min, many times a maximum of 60 min, and in some cases a maximum of 10 min is necessary to detect by means of samples that the fluorescence has largely been quenched or has been quenched to the extent that is desired in the individual case. The quenching of the fluorescence [determined] by means of a sample indicates that the fluorescent compound that is to be oxidized has been oxidatively cleaved at at least two fracture sites. This can be demonstrated by TLC or HPLC.

To conduct the method in accordance with the invention, the aqueous system is mixed with a solution of an oxidase or peroxidase and with one of the said oxidation agents. The concentration of the fluorescent compound here can lie in the range from 3 parts per billion (ppb) up to 1 wt% and higher, for example when one is working with a concentrated medium containing a highly fluorescent compound.

The amount of enzyme to be used is governed by the content of the fluorescent compound in the aqueous system. In general the peroxidase is used in an amount of $1\text{-}10^7$ PODU (= peroxidase units) per g of fluorescent compound. The oxidase is used in an amount of $1\text{-}10^7$ laccase unit per g of fluorescent compound. The term PODU is understood to be the amount of enzyme that, under standard conditions (0.1M phosphate buffer, pH = 7, temperature = 30°C, 0.88mM H_2O_2, 1.67mM ABTS), catalyzes the conversion of 1 µmol H_2O_2 in 1 min in a system in which ABTS (2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) was oxidized. The term laccase unit is understood to be the amount of enzyme that, under stand conditions (0.1M sodium acetate buffer, pH = 5, temperature = 30°C, oxygen), catalyzes the conversion of 1 µmol ABTS per minute. Under these standard conditions a green-blue dye is produced, which can be measured photometrically at 418 nm.

In the case of the absorption of a conventional usage amount of 1 wt% brightener on bone dry (bd) paper the enzyme dosage necessary to eliminate the fluorescence is, for example, $10\text{-}10^8$ units per kg bd paper, preferably $100\text{-}10^6$ units per kg bd paper. This corresponds to an amount of 1 µL to 1000 mL enzyme solution per kg bd paper, preferably 10 µL to 100 mL per kg bd paper, if the enzyme solution that is used has an activity of 10,000 units/g. A corresponding recalculation must be made if the content in units/g varies from this.

The H_2O_2 concentration in the aqueous system to be treated is in general 0.01 mmol to 2.0 mol, preferably 0.1 mmol to 1 mol, per liter. With respect to bone dry material that is present in the aqueous system to be treated, chiefly 0.001-5 wt% H_2O_2 is used. If one wishes to gear the amount to the amount of fluorescent compound, the amount of H_2O_2 will be 1×10^{-5} to 1×10^{-2} wt% with respect to the fluorescent compound. If O_2 is used as oxidation agent, one can operate with pure oxygen, but atmospheric air or atmospheric air enriched with oxygen can also be used; since the O_2 concentration of atmospheric air is normally sufficient in the case of laccase systems, use is made of this inexpensive possibility. For an aqueous system contact time of 0.1-100 min at pH 7 and 37°C no fluorescent compound that can be detected from its fluorescence is detectable. The reaction time can be shifted toward shorter times by the appropriate increase of the amount of enzyme.

If the aqueous system to be treated is a suspension or slurry that accordingly has a solids content this solids content amounts to 0.1-30 wt%, preferably 0.5-10 wt%, of the total suspension or slurry.

In cases in which the fluorescent compounds are in adsorbed state on a solid surface, it is advantageous to put the fluorescent compound into solution first. Substantially known anionic surfactants or, if cellulose is the substrate, especially cellulases or endoglucanases, are suitable for this.

The progress and completeness of the oxidative decomposition of the fluorescent compound can be followed by measuring the fluorescence. For this a sample of the aqueous system (wastewater or dispersion or aqueous slurry) can be input directly into a measurement apparatus; however, chromatographic methods for separating the substances present in the aqueous system can also be used. After the end of the reaction the enzyme can be deactivated by simply changing the pH and/or increasing the temperature. In closed circulation cycles this is generally not necessary, so that here it is only necessary to add additional enzyme as the enzyme activity decreases. To interrupt the enzyme activity, it is also possible to add catalases. In another embodiment one can also make use of the immobilization of enzymes that is well known to the specialist, where a support material with enzymes in immobilized form is arranged, for example in a column, and brought into contact with the aqueous system that is to be treated; such an embodiment is, of course, applicable only to dissolved fluorescent compounds and not to dispersions or slurries.

The method in accordance with the invention needs a considerably smaller amount of oxidation agent and auxiliary agent and enables faster and more environmentally friendly elimination of fluorescent compounds. In comparison, the chemical requirements for a nonenzyme-catalyzed decomposition using potassium peroxodisulfate is 1-2 wt% persulfate with respect to the solids (for example pulp) for a pH of 10.5 and 60°C, a stock consistency of 5 wt% and a treatment time of 180 min. In the case of treatment with a chlorine alkali at pH = 3 the use of 0.5 wt% active chlorine with respect to solids (for example pulp) is necessary in order to achieve a quenching of fluorescence at 60 min for a stock consistency of 2.5 wt%. When cationic polymer auxiliary substances are used, up to 2 wt% of the auxiliary substance is used in order to achieve quantitative quenching for 0.8 wt% fluorescent compound. Decomposition does not take place in this case, or it occurs only in a subordinate amount.

The fragments obtained through the enzymatic decomposition of the fluorescent compounds as a rule are more susceptible to microbiological decomposition in a wastewater treatment plant than the untreated fluorescent compounds.

All percentage data refer to percent by weight.

Example 1

A stock solution with a concentration of 0.05% (25% solution) of a brightener of formula (A) (Example 12 of EP 0 409 028), see above formula listing, was prepared in a 50mM

potassium phosphate buffer (pH 7). The sample was incubated with 500 units of horseradish peroxidase (Fluka, No. 77333) at 28°C. The enzyme had an activity of 700 units/mg. 1 unit oxidizes 1 µmol ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) per minute at 25°C and pH 6.

The concentration of the peroxidase was 100 units/µL of brightener (calculated for 25% as delivered). The hydrogen peroxide concentration was 0.15 mmol/L. After various times samples were taken from each test batch, centrifuged, and the absorption and fluorescence was tested.

The absorption spectrum was determined with a spectrophotometer in the 200-500 nm wavelength range. The emission was measured at 440 nm by means of fluorescence spectroscopy at an excitation wavelength of 280 or 350 nm.

Conditions for thin layer chromatographic separation

In addition, the test solutions were investigated by RP-TLC (reversed phase thin layer chromatography):

0.25 mm silica gel (stationary phase with fluorescence indicator, RP 18 W/UV 254, Macherey-Nagel),

Aqueous eluent containing 8.75% (w/v) oxalic acid and 0.14% (w/v) sodium heptane sulfonate/methanol/acetonitrile (4:1:1) in water.

Results

During the enzyme treatment (21 h) the intensity of the absorption band at 350 nm decreased by 72%. Only a low emission at 440 nm could be seen after the enzyme treatment; this emission is due to stimulation at wavelength 280 or 350 nm in the case of an intact brightener molecule. The fluorescence was weakened by 90-95%.

The stilbene derivative that elutes as a spot at Rf = 0.83 was converted to three new spots by enzyme treatment, which eluted at the Rf values 0.875/0.765/0.73. After less than 200 min the spot of the educt could no longer be seen.

Example 2

A stock solution with a concentration of 0.05% (a 25% solution, v/v) of a brightener of formula (A) was prepared in 50mM potassium phosphate buffer (pH 7). The sample was incubated with 10 units of a peroxidase (Novozym 502, Novo Nordisk) at 37°C. The enzyme is a recombinant heme-containing enzyme, which was originally isolated from Basidiomycetes and had an activity of 10,000 PODU/g. 1 PODU oxidizes one µmol ABTS (2,2'-azinobis(3-

ethylbenzothiazoline-6-sulfonic acid, c = 1.67mM) per minute at 30°C, pH 7 and an H_2O_2 concentration of 0.88mM. The enzyme additionally has a secondary activity as amylase.

The concentration of the peroxidase was 7 units/μL of brightener (calculated for 25% supplied form). The hydrogen peroxide concentration (use 30%) was 0.15 mmol/L. After various times samples were taken from each test batch, centrifuged, and their absorption and fluorescence were investigated as in Example.

Results

The enzyme treatment led to a reduction of the absorption at 350 nm by about 90%. The emission at 440 nm, which is measured with stimulation at wavelength 280 nm or 350 nm, was weakened by 95-98%.

The stilbene derivative that was eluted without enzyme treatment (100 min) as a spot at $R_f = 0.83$, was split by enzyme treatment so that three new spots eluted at R_f values 0.875, 0.765, and 0.73. After 100 min the spot of the educt could no longer be seen. After 100 min, therefore, the brightener had been completely fragmented.

Example 3

Example 1 was repeated except that peroxidase from Example 2 in a concentration of 700 units per μL brightener was used. After a few seconds the brightener was completely fragmented.

Example 4, Controlled experiment

A corresponding experiment by analogy with Example 1, in which only hydrogen peroxide, but no enzyme, was used, did not lead to a decrease of the fluorescence under identical conditions. Control by thin layer chromatography gave only one spot, which was assigned to the brightener.

Example 5 (Application example)

100 g base paper consisting of 70% bleached pine sulfate and 30% bleached birch sulfate pulp that contained 0.25% active agent in accordance with the above formula (A) and had a CIE whiteness of 125 was beaten to a pulp consistency of 5% (2000 mL, 3000 rpm).

First 7000 endoglucanase units (EGU) Cellusoft L were added (10 h, 50°C).

30,000 PODU (3 mL), determined in accordance with Example 2, was of the peroxidase from Example 2 was dispensed at 37°C and pH 6. Then hydrogen peroxide (0.15 mol/L of solution) was added and the mixture was held for 120 min at 37°C; the isolated pulp was partially degraded.

After the end of the enzyme treatment the fiber slurry was diluted to a stock consistency of 0.5% and a paper sheet was made on a Rapid Kothen sheet maker. The paper sheet had a CW whiteness of 85. A nonbleached paper usually has a whiteness degree of 80. The above value of 85 accordingly shows the decomposition of the brightener.

Example 6, Comparison Example

The brightener quenching can also be achieved if a 0.5% solution of brightener of formula (A) is treated with 2% potassium peroxodisulfate for 120 min at pH 10.5/60°C. The conditions for degradation of the brightener are, however, considerably more drastic (pH, temperature) and require a higher amount of oxidation agent with a comparably long time.

Claims

1. A method for oxidative decomposition of fluorescent compounds in aqueous systems, which is characterized by the fact that such aqueous systems are treated with at least enzyme from the group of the oxidases and peroxidases, which can be present both as such by themselves or also with at least one other enzyme that has hydrolytic activity, and with an oxidation agent from the group of O_2, H_2O_2, and compounds that contain or form O_2 or H_2O_2, at a temperature from 5-90°C and a pH value from 4-10, where the treatment is carried out in the absence or in the presence of surface-active auxiliary agents.

2. A method as in Claim 1, which is characterized by the fact that the enzyme with hydrolytic activity that is considered for combining with the oxidases and peroxidases is at least one from the group of the enzymes that degrade cellulose or hemicellulose, lipases, amylases, proteases and dehalogenases.

3. A method as in Claims 1 and 2, which is characterized by the fact that the combined enzyme that has hydrolytic, cellulose-, or hemicellulose-decomposing activity is cellulase or xylanase or a mixture of the two.

4. A method as in Claim 1, which is characterized by the fact that the treatment is carried out at 10-60°C, preferably 20-55°C.

5. A method as in Claim 1, which is characterized by the fact that the treatment is carried out at a pH value of 5-8.

6. A method as in Claim 1, which is characterized by the fact that the aqueous system to be treated is wastewater or circulating water from the treatment or the production of cellulosic material or a suspension of cellulosic material.

7. A method as in Claim 6, which is characterized by the fact that the cellulosic material is paper, cardboard or pasteboard.

8. A method as in Claim 1, which is characterized by the fact that the fluorescent compounds to be decomposed contain 4,4'-diaminostilbenedisulfonic acid, distyrylbenzene, distyrylbiphenyl, stilbenyl-2H-triazole, benzoxazole, benzofuran, or coumarin as molecular backbone.

9. A method as in Claim 8, which is characterized by the fact that the fluorescent compounds to be decomposed contain 4,4'-diaminostilbenedisulfonic acid or distyrylbiphenyl, preferably 4,4'-diaminostilbene disulfonic acid, as molecular backbone.